Different combinations of perennial ryegrass and white clover phenotypes do not affect mixture yield under cutting management during establishment

L. ROSSI¹, D.F. CHAPMAN² and G.R. EDWARDS³

¹ DairyNZ, Private Bag 3221, Hamilton 3240, New Zealand

² DairyNZ, P.O. Box 160, Lincoln University, Lincoln 7647, New Zealand

³ Agriculture and Life Sciences Faculty, Lincoln University, P.O. Box 84, Lincoln University, New Zealand
laura.rossi@dairynz.co.nz

Abstract

A field experiment was conducted for 12 months under irrigation and cutting management to determine if interactions between perennial ryegrass and white clover cultivars of different phenotypes could affect pasture yield and botanical composition during establishment. Four ryegrass and four clover cultivars, differing in leaf and tiller/stolon traits, were grown in all combinations (n=16), along with monocultures of each (n=8), as sub-plots under two nitrogen fertiliser levels (100 or 325 kg N/ha/year). Dry matter yield and botanical composition were measured on nine occasions and ryegrass and clover population densities were determined four times. Total annual yield was similar for all mixture combinations due to substitution between the sward components. While there were significant yield differences among ryegrass or clover cultivar monocultures, these seldom explained differences in mixture yields. Mixtures yielded more DM than ryegrass monocultures under both N treatments (+1.3 to +3.9 t DM/ha/year).

Keywords: perennial ryegrass, white clover, dairy, dry matter yield, nitrogen fertiliser, phenotype, competition

Introduction

Perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) are the main components of pastures in New Zealand. Generally, when ryegrass and clover cultivars of different phenotypes are grown in a mixture the total annual dry matter (DM) yield of the pasture is similar, although differences in botanical composition can occur (Reid 1961; Connolly 1968; Camlin 1981; Rhodes & Harris 1979; Widdup & Turner 1983; Ledgard *et al.* 1990). More ryegrass and clover cultivars with a greater phenotypic range are now available than when these above studies were undertaken. This raises the question as to whether interactions between modern cultivars can affect pasture yield and botanical composition to provide more productive pastures. This study addresses this question and examines how grass and clover characteristics may affect their competitive

ability. Another objective was to quantify the effect of the inclusion of clover on pasture dry matter (DM) yield and its implications for ryegrass cultivar performance. The working hypothesis was that the DM production of the sward will not differ when modern perennial ryegrass and white clover cultivars with different phenotypes are grown in mixtures. All combinations of four perennial ryegrass and four white clover cultivars grown in binary mixtures at different nitrogen (N) fertiliser levels were compared. The perennial ryegrass cultivar by white clover cultivar interaction term was considered the critical test of the hypothesis. Monocultures of all cultivars were also included to help separate the effects on mixture yields of variation among cultivars in yielding ability *per se* from phenotypic factors such as tiller or stolon density and leaf morphology.

Methodology and analysis

The experiment was sown in November 2013 at the Lincoln University Research Dairy Farm, Lincoln, Canterbury, New Zealand (latitude 43°38'10.26"S; longitude 172°27'42.91"E; altitude 12 m a.s.l.) and measurements were made from 1 June 2014 to 31 May 2015. Soils at the site were Paparua sandy loam and Wakanui sandy loam, typic immature pallic soils and mottled immature pallic soils, respectively, (New Zealand soil classification, Hewitt 2010). Total rainfall during the experiment (376 mm) was 223 mm lower than the 30 year average (1981 to 2010) of 599 mm. Mean temperature for the period (12.3°C) was 0.7°C higher than the historic average (National Institute of Water and Atmospheric Research 2015).

The experimental design was a split-plot with four replicate blocks. Main plots were two N levels (100 and 325 kg N/ha/year), randomised within blocks. Subplots were the pasture types (24), made up of a 4 × 4 factorial of four perennial ryegrass cultivars and four white clover cultivars (16 subplots), plus monocultures of each cultivar (8 subplots), randomised within main plots. Subplots were 3 x 1.8 m; from within that area, the 10 central drill lines (1.5 m width) were harvested, resulting in a measurement area of 4.5 m². Four

Table 1 Total DM yield (kg DM/ha) from pastures sown with mixtures of perennial ryegrass and white clover cultivars and from monocultures. Data are means of two N fertiliser rates.

Month										

SED = standard error of the difference between means. Different letters within a column indicate statistical differences. n.m. not measured. † High clover yields in Oct-14 reflect the fact that this was the first harvest applied to clover monocultures and included herbage accumulation throughout early spring.

perennial ryegrass cultivars were compared providing a range from fine to broader leaved and from open to denser plant habits. The cultivars were Abermagic AR1 (a dense, fine-leaved, diploid), Arrow AR1 (a dense, medium to broad-leaved, diploid), Prospect AR37 (a dense, medium to wide-leaved, diploid) and Bealey NEA2 (an open, medium-leaved, tetraploid). The four white clover cultivars evaluated represented a range in leaf size: Nomad (small-leaved), Bounty (medium-leaved), Tribute (medium-large-leaved) and Kopu II (large-leaved).

Sowing rates were 20 kg/ha of seed for the diploid ryegrasses and 28 kg/ha for the tetraploid ryegrass to account for differences in seed weight between the ploidy levels. White clover was sown at a rate of 4 kg/ha of bare seed (correction of this sowing rate was made to account for seed coating).

Defoliation was by cutting, to avoid confounding animal effects; irrigation was applied from October 2014 to March 2015 (400 mm).

N fertiliser was applied manually as urea (46-0-0). In the Low N (100 kg N/ha) treatment, urea was applied at rates of 25 kg N/ha on four occasions. In the High N (325 kg N/ha) treatment, it was applied at a rate of 35.2 kg N/ha on six occasions and at a rate of 57 kg N/ha for the last two applications.

T-Max™ (30 g/L aminopyralid) at 60 ml/10 L water was applied on 3rd January 2015, with a knapsack sprayer, to the perennial ryegrass monocultures to control white clover and other legumes. The same day Gallant™ Ultra (520 g/L haloxyfop-P) at 12 ml/10 L water with Uptake™ spraying oil (582 g/L paraffinic oil and 240 g/L alkoxylated alcohol non-ionic surfactants) at 15 ml/10 L water were applied with a knapsack sprayer to the white clover monocultures to control perennial ryegrass and other grasses.

Total DM yield was estimated on nine occasions (the first on the 22 August 2014 and the last on 12 May 2015) by harvesting the entire 4.5 m² measurement area to 5.5 cm above ground level, using a Haldrup forage harvester (Haldrup F-55, Denmark). Botanical composition was determined by dissecting a 15 g (fresh weight) subsample into: live perennial ryegrass, live white clover, live other species and dead material of all species. Components were dried for 72 hours at 65°C. Perennial ryegrass and white clover population densities were measured during June 2014, November 2014, January 2015 and May 2015; a 5 × 20 cm (100 cm²) frame was randomly positioned at three locations in each subplot and the number of perennial ryegrass tillers and white clover growing points within each frame were counted.

Statistical analyses were conducted for mixtures, monocultures of perennial ryegrass and monocultures of white clover, using ANOVA in GenStat 17 (VSN

International 2014). Repeated measurements analyses were conducted on the total DM yield using the AREPMEASURES procedure in GenStat 17 (VSN International 2014). Perennial ryegrass and white clover yields (kg DM/ha) within the mixture treatments were analysed by ANOVA (VSN International 2014). These yields were calculated based on the total DM and botanical composition data for each harvest. For the white clover content of mixtures (% DM), the repeated measurements through time were analysed using spline models within the linear mixed model framework as described by Verbyla *et al.* (1999). Regression analyses were conducted between perennial ryegrass and white clover population density, population density and DM yield and between DM yields for monocultures and mixture.

Results

No interactions between perennial ryegrass and white clover cultivars on DM yield of the mixtures were observed, with one exception (Table 1). In November 2014, there was an interaction between N treatment, perennial ryegrass cultivar and white clover cultivar (P=0.045). Mixtures including Arrow AR1 and Kopu II grown under the High N treatment yielded less than the same mixtures under the Low N treatment. Mixtures containing Abermagic AR1 were the highest yielding irrespective of the N treatment and white clover cultivar included.

The effect of perennial ryegrass cultivar on the DM yield of the mixture was significant in six of the nine harvests (Table 1). Mixtures sown with different ryegrass cultivars were variable in their production, and the highest yielding mixture differed between harvests. Thus, total annual DM yield was similar irrespective of the perennial ryegrass cultivar included (P=0.089). White clover cultivar effect on the DM yield of a mixture was significant on only three occasions, resulting in a similar annual DM yield for the mixtures with different white clover cultivars (P=0.670).

Mixed pastures yielded 16.4% more than perennial ryegrass monocultures (+ 2.6 t DM/ha, average of both N treatments); however, the increment due to inclusion of clover varied when the pastures were grown under the different N treatments (N × white clover presence interaction, P<0.001). Mixtures with low N application rate yielded 30.6% more DM/year than perennial ryegrass monocultures with the same level of N (+ 3.9 t DM/ha), while mixed pastures yielded only 6.8% more than perennial ryegrass monocultures (+1.3 t DM/ha) with high N application (Table 2).

Apart from the first harvest of the season (August 2014; data not presented) the white clover content (% DM) of mixtures was greater under the Low than under

Figure 1 White clover content (% of DM) of mixtures sown with different white clover cultivars (bars indicate SED - standard error of differences between means). Bounty (□ means raw data — spline curve), Kopu II (○ means raw data spline curve), Nomad (▼ means raw data - - - spline curve), Tribute (△ means raw data ---- spline curve). Data are means of two N levels.

the High N treatment The effect of perennial ryegrass cultivar on clover content was greater in spring (August 2014, $P<0.001$; October 2014, $P=0.003$) and autumn (May 2015, $P=0.001$) whilst the effect of white clover cultivar occurred during most of the year (Figure 1). However, clover content was affected by the interaction between perennial ryegrass and white clover cultivar only once (May 2015, $P=0.005$).

Ryegrass cultivars differed in tiller density at all four sampling times. The tetraploid, Bealey NEA2, had the lowest tiller density (no./m^2) both in monoculture and mixture. Mixed pastures had fewer tillers/ m^2 than the perennial ryegrass monocultures in June 2014 ($P<0.01$) and May 2015 ($P<0.001$), but not in November 2014

or January 2015 ($P=0.220$ and 0.334 , respectively). Significant interaction between grass and clover cultivar on tiller density was detected only in November 2014 ($P=0.023$).

Differences between clover cultivar monoculture growing point densities were significant at all four sampling times, with Bounty and Nomad most consistently having the most growing points/ m^2 . However, in mixture the effect of clover cultivar on growing point density only occurred in June 2014 ($P=0.003$). During the entire season, the number of clover growing points/ m^2 was higher in monocultures than in mixtures.

Discussion

Mixture yields: interactions between grass and clover cultivars

Except for one date, no interactions between perennial ryegrass and white clover cultivars on DM yield of the mixtures occurred (November 2014). Thus, the hypothesis proposed is supported and breeding of both perennial ryegrass and white clover over recent decades has not altered the conclusion previously drawn, that interactions between perennial ryegrass and white clover cultivars do not affect the total yield of mixtures. This study found that monocultures of perennial ryegrass and white clover grew at similar rates from November to March (Table 1) and produced similar

total annual DM yields, especially under low N application rate (Table 2). This suggests that substitution between the two pasture species largely negated any effects of grass cultivar by clover cultivar interactions on the total yield of binary mixtures.

Mixture yields: main effects of grass and clover cultivar

While grass cultivar by clover cultivar interactions

in total mixture yield were seldom observed, significant differences in the yield of mixtures based on different ryegrass cultivars or different clover cultivars occurred in six and three of the nine months, respectively (Table 1). Perennial ryegrass dominated botanical composition of the mixtures throughout the study. White clover only contributed 50% or more of total pasture DM in January, February and March for pastures based on Bounty and Kopu II in the Low N treatment (data not presented; Figure 1 shows general trends in clover content). Hence, it is not surprising that significant differences in the yield of the mixtures differing in perennial ryegrass cultivar were observed more often than differences due to white clover cultivar. These findings agree with Camlin (1981) who compared perennial ryegrass/white clover mixtures fertilised with 200-240 kg N/ha/year and observed that mixture yield tended to reflect the yield of the grass component.

The relative yields of mixtures based on different ryegrass cultivars aligned with relative differences in monoculture yields on only two of the six occasions (August and November) when ryegrass cultivar influenced mixture yields. Regression analyses confirmed that, in these 2 months, there was significant positive association between the mean perennial ryegrass cultivar yield in monoculture and in mixture (August: $P=0.006$; $R^2=0.989$; November: $P=0.002$; $R^2=0.997$). Both times, the relative yield of Abermagic AR1 strongly influenced the outcomes. Abermagic AR1 yielded markedly less than the three other ryegrass cultivars in August, but markedly more in November (Table 1). In the other 7 months of the year, perennial ryegrass cultivar yields in monoculture were similar. Meanwhile, tiller density differed consistently among the ryegrass cultivars. Hence, if ryegrass phenotypic characters such as tiller density exert sufficient influence on grass/clover interactions to alter the yield of mixtures, there was ample opportunity for this effect to emerge without any confounding effect of cultivar growth rate *per se*, but it did not eventuate.

Applying the same analysis to white clover, clover yields in monoculture aligned with differences in mixture yield based on the different clover cultivars on only one occasion (May 2015). Then, the large-leaved and medium-large leaved cultivars, Kopu II and Tribute, respectively, yielded more DM in monoculture than the small-leaved and medium-leaved cultivars Nomad and Bounty, respectively. This flowed through to relative yields of the mixtures based on the four cultivars, notably in the contrast between Tribute and Bounty (Table 1). Otherwise, there was opportunity for the consistent differences in stolon and leaf characters of the white clover cultivars observed throughout the study to influence mixture performance, but again, this did not eventuate.

Different conditions for pasture growth were created by the use of Low and High N application rates. N availability affects competition between grass and clover in mixtures. However, in general, no interactions between N and perennial ryegrass cultivar or N and white clover cultivar were detected.

Mostly, the causes of differences in mixture yields remain unexplained. While there were several statistically significant differences, in quantitative terms they were small and agronomically unimportant. The largest occurred in mixtures based on different ryegrass cultivars in August and November (c. 1 and 0.5 tonne DM/ha, respectively) and, as described above, were explained by the yield of Abermagic AR1 relative to the other cultivars in monoculture.

However, these conclusions are drawn from only 1 year of data (covering the period 6 to 18 months post-sowing) from pastures managed by cutting. Caution is therefore required when extrapolating to longer time frames and to grazing. Most of the clover plants would have been in the tap-rooted phase of development that usually lasts between 1 and 2 years after sowing (Brock *et al.* 2000; Brock & Hay 2001), and in which the legume tends to produce the greatest amount of DM per unit area (Widdup & Barrett 2011). Therefore, the high clover yields observed here may not be present in established pastures when clover plants move into the clonal phase. The relationship between ryegrass and clover changes as mixed swards mature and factors such as defoliation frequency and height, and the addition of N to the system through clover fixation, can all modify competition between the species (Harris & Thomas 1973; Schwinning & Parsons 1996a, 1996b). Other aspects of the grass/clover association, such as pasture nutritive value and animal production (e.g. Crush *et al.* 2006), also need to be considered. Some of these aspects are being investigated by continuing measurements on the experiment into the third year post-sowing.

Conclusions

The finding that substitution between species led to similar yield of mixtures based on different binary combinations of perennial ryegrass and white clover cultivars agrees with previous research. It also reaffirms the statement of Stewart (2006): lifting overall pasture production from mixtures is challenging because ‘... any increase in the ryegrass yield is often partially cancelled by decreased clover yields’.

ACKNOWLEDGEMENTS

The study was funded by New Zealand dairy farmers through DairyNZ Inc., project number RD 1414. Thanks to DairyNZ science and technical teams, Lincoln University, Lincoln University Research Dairy

Table 2 Annual DM yield (kg DM/ha) of monocultures and mixture grown under High or Low N treatment, and apparent response to N (kg DM/kg N).

	Low N	High N	Apparent response to N (kg DM/kg N) ¹
Perennial ryegrass monoculture	12665	18650	21.0
White clover monoculture	14420	14565	0.5
Mixture - total ²	16540	19920	11.9
Mixture - perennial ryegrass component	10795	16635	20.5
Mixture - white clover component	4880	2135	-9.6

¹ Calculation based on a difference of 285 kg N/ha/year (instead of 225 kg N/ha/year) between the High and the Low N treatment due to the extra fertiliser applied to both treatments to replace the N removed.
² The difference between the total mixture yield and the sum of perennial ryegrass and white clover components is explained by the presence of other species and dead matter.

Farm staff, Field Service Centre staff and postgraduate students. Thanks to Barbara Dow and Alison Lister for their assistance with statistical analyses. Helpful review comments on the manuscript from Warwick Harris and Keith Widdup are gratefully acknowledged.

REFERENCES

- Brock, J.L.; Albrecht, K.A.; Tilbrook, J.C.; Hay, M.J.M. 2000. Morphology of white clover during development from seed to clonal populations in grazed pastures. *Journal of Agricultural Science* 135: 103-111. doi:10.1017/s0021859699008060
- Brock, J.L.; Hay, M.J.M. 2001. White clover performance in sown pastures: a biological/ecological perspective. *Proceedings of the New Zealand Grassland Association* 63: 73-83.
- Camlin, M.S. 1981. Competitive effects between ten cultivars of perennial ryegrass and three cultivars of white clover grown in association. *Grass and Forage Science* 36: 169-178. doi:10.1111/j.1365-2494.1981.tb01553.x
- Connolly, V. 1968. A comparison of six white clover and three perennial ryegrass varieties under a system of grazing management. *Irish Journal of Agricultural Research* 7: 227-242.
- Crush, J.R.; Woodward, S.L.; Eerens, J.P.J.; MacDonald, K.A. 2006. Growth and milksolids production in pastures of older and more recent ryegrass and white clover cultivars under dairy grazing. *New Zealand Journal of Agricultural Research* 49: 119-135.
- Harris, W.; Thomas, V.J. 1973. Competition among pasture plants III. Effects of frequency and height of cutting on competition between white clover and two ryegrass cultivars. *New Zealand Journal of Agricultural Research* 16: 49-58.
- Hewitt, A.E. 2010. New Zealand soil classification (3rd Ed.). Lincoln, New Zealand: Manaaki Whenua Press, Landcare Research.
- Ledgard, S.F.; Brier, G.J.; Upsdel, M.P. 1990. Effect of clover cultivar on production and nitrogen fixation in clover-ryegrass swards under dairy cow grazing. *New Zealand Journal of Agricultural Research* 33: 243-249.
- National Institute of Water and Atmospheric Research. (2015). CliFlo: The National Climate Database. Retrieved from <http://cliflo.niwa.co.nz/>
- Reid, D. 1961. Factors influencing the role of clover in grass-clover leys fertilized with nitrogen at different rates. II. The effects of the variety of white clover on the yields of total herbage and clover. *Journal of Agricultural Science* 56: 155-160.
- Rhodes, I.; Harris, W. 1979. The nature and basis of differences in sward composition and yield in ryegrass-white clover mixtures. *British Grassland Society, Occasional Symposium* 10: 55-60.
- Schwinning, S.; Parsons, A.J. 1996a. Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. *Journal of Ecology* 84: 799-813. doi:10.2307/2960553
- Schwinning, S.; Parsons, A.J. 1996b. A spatially explicit population model of stoloniferous N-fixing legumes in mixed pasture with grass. *Journal of Ecology*, 84: 815-826. doi:10.2307/2960554
- Stewart, A.V. 2006. Genetic origins of perennial ryegrass (*Lolium perenne*) for New Zealand pastures. Proceedings of the 13th Australasian Plant Breeding Conference. pp. 11-20.
- Verbyla, A.P.; Cullis, B.R.; Kenward, M.G.; Welham, S.J. 1999. The analysis of designed experiments and longitudinal data by using smoothing splines. *Journal of the Royal Statistical Society: Series C (Applied Statistics)* 48: 269-311. doi:10.1111/1467-9876.00154
- VSN International. 2014. GenStat for Windows 17th Edition [Computer software]. Hemel Hempstead, UK: VSN International.
- Widdup, K.H.; Barrett, B.A. 2011. Achieving persistence and productivity in white clover. *Grassland Research and Practice Series* 15: 173-180.
- Widdup, K.H.; Turner, J.D. 1983. Performance of 4 white clover populations in monoculture and with ryegrass under grazing. *New Zealand Journal of Experimental Agriculture* 11: 27-31.

Reducing nitrogen fertiliser alters dairy shed effluent quality

S.J. DENNIS

Grounded, Hardys Road, RD 1 Colgate 7673, New Zealand
samual@grounded.co.nz

Abstract

Dairy farmers using low rates of nitrogen fertiliser observed reduced odour in milking sheds, and a smaller growth response of pasture to effluent application. Effluent samples from four conventional (>100 kg N/ha/year, “high-fert”) and three low-nitrogen (N) fertiliser (<50 kg N/ha/year, “low-fert”) properties were collected in January 2017 and analysed for total N concentration, N form, mineral nutrient concentration and pH. Total effluent N concentration was comparable between both classes of farm. However, low-fert properties had a higher proportion of N in organic forms as opposed to ammoniacal-N than high-fert properties (mean 75% and 59% organic on low- and high-fert properties, respectively, $P < 0.01$). Low-fert effluent also had a lower pH, higher P concentration, and nearer optimal N:P ratio than effluent from high-fert properties. It was hypothesised that reducing N fertiliser may result in more nutritionally-balanced effluent (N:P ratio), causing microbes to multiply more rapidly (lowering pH), storing N in microbial biomass, reducing ammonia emissions and odour, and reducing the risk of N leaching from effluent applied to pasture.

Keywords: dairy shed effluent, nitrogen fertiliser, ammonia, organic N, environmental loss

Introduction

Dairy shed effluent is a valuable source of nutrients for pasture plants on dairy farms (DairyNZ 2015). However, it is also a potential source of nutrient loss predominately via the following pathways: ammonia volatilisation (Li *et al.* 2014), nitrate leaching, and phosphorus runoff. Effluent is highly variable, and the characteristics of effluent will alter the quantity of nutrients available for loss via each pathway, and therefore the potential for environmental losses (Houlbrooke *et al.* 2011).

Nitrogen (N) exists in effluent in both inorganic (ammonium (NH_4^+) and nitrate (NO_3^-)) and organic forms. Inorganic N is susceptible to gaseous loss as ammonia (NH_3) and leaching as nitrate. Although dissolved organic nitrogen can leach, as a general rule, organic nitrogen is unlikely to be lost unless it is first converted to inorganic forms (Houlbrooke *et al.* 2011).

Inorganic nitrogen is available to plants and can be rapidly used for growth. Therefore, effluent rich

in ammonia and/or nitrate is likely to result in an immediate plant growth response. However, organic nitrogen (other than urea) is slowly released for plant use, and slow-release nutrients may result in a higher long-term pasture growth response per unit of N applied than soluble nutrient applications (Zaman *et al.* 2009).

Farm staff on properties in the study group using low rates of N fertiliser noticed a reduced odour in the milking shed, and also a reduction in the immediate visible pasture response to effluent application, compared with their expectations from past experience under conventional management (A. Lapping pers. comm.). This paper describes the results from a farmer-funded effluent analysis study designed to determine whether these observations were due to actual differences in the characteristics of effluent between farms using low or high rates of synthetic N fertiliser.

Methodology and analysis

Seven dairy farms were chosen for this study. All were located on free-draining alluvial soil and annual rainfall was supplemented with irrigation. All farms were located between Greendale and Dunsandel, in Canterbury, New Zealand. On most farms herd size was 500-800 cows (one “low-fert” farm had 300 and one “high-fert” farm had 1500). All used twice-a-day milking at the time of sampling, most farms used a daily acid wash and two alkali washes per week (one high-fert farm used only one alkali wash per week). Insufficient data were available on wash-down water use to determine whether dilution differed between farms. Three farms applied low total rates of N fertiliser (30-43 kgN/ha/year, as both organic and synthetic forms), and four applied moderate to high rates (100-350 kgN/ha/year, primarily as urea). These two categories were referred to as low-fert and high-fert farms, where “fert” refers solely to nitrogen. Supplementary feed practices were comparable, with cows receiving a grain or PKE supplementary feed on most properties, although two low-fert and one high-fert property also fed seaweed and/or fish hydrolysate as an animal nutritional supplement.

Eleven effluent samples were collected on two dates in January 2017. Four farms (two low-fert, two high-fert) were sampled on 16 January. All seven farms were sampled on 30 January. Samples were